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Protein and RNA quantification of multiple genes in single cells

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ABSTRACT

Single-cell analysis overcomes the problems of cellular heterogeneity by revealing the individual differences between cells in tissue. The current tools used to profile gene expression at the single-cell level are arduous and often require specialized equipment. We have previously developed a technique to quantify protein expression levels in single living cells. Here, we combine quantification of protein expression with absolute measurement of mRNA amounts of the same gene in the same cell, to profile the expression of genes at the transcriptional and translational levels. We show that high heterogeneity exists at both the mRNA and protein levels for multiple genes, even among monoclonal cells. We demonstrate a rapid, straightforward approach to single-cell profiling of RNA and protein production.

METHOD SUMMARY

We report a fast and reliable technique for the quantification of endogenous protein and mRNA levels of any gene from single cells.

KEYWORDS

CRISPR-Cas9 • fluorescence imaging • genome editing • mRNA quantification • protein quantification • single-cell analysis

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BioTechniques 66: 15-21 (January 2019)
10.2144/btn-2018-0130

First draft submitted: 4 September 2018; Accepted for publication: 27 September 2018

INTRODUCTION

Understanding the relationship between genes and phenotypes is a central component of biology. Profiling the expression of genes with respect to mRNA or protein products has advanced our fundamental understanding of cell biology, such as the maintenance of cell structure, progression of cell cycle and neural development, as well as how abnormalities in gene expression can lead to disease states. Several analytic methods have been developed to profile gene expression at the level of mRNA or protein. For example, microarrays and RNA sequencing approaches can identify mRNA from tens of thousands of different genes whereas protein expression can be assayed using spectrometry, chromatography, immunoassays and gel electrophoresis [1,2].

The current tools to assay mRNA and protein expression are generally performed on large pools of cells or tissues. Large numbers of cells can increase the reliability of results and overcome issues of low sensitivity or the low signal-to-noise ratio of common assays. However, it is becoming increasingly clear that even neighboring cells within tissues or pools of clonal cells are not homogenous [3–8]. Cellular dynamics, such as differing transcriptional and translational states, cell signaling, cell cycle, development, and other molecular processes in addition to stochasticity together produce cellular heterogeneity, even in immortalized clonal cell lines. Assays performed on large numbers of cells result in population-averaged measurements that miss important individual cellular differences, and the results obtained from population samples cannot be translated into the single-cell scale. Therefore, analysis of single cells is required to identify hidden molecular mechanisms that are masked by the population average.

We have previously developed a technique that allows for quantitation of protein levels in single cells *in vivo* [5]. This protein quantitation ratioing (PQR) technique uses a genetic tag that produces a stoichiometric ratio of a fluorescent protein reporter and the protein of interest during protein translation. The fluorescence intensity is proportional to the number of molecules of the protein of interest produced and is used to determine the relative protein amount within the cell (Figure 1A). Using genome editing tools, PQR constructs can be inserted into any endogenous genomic locus to quantify endogenous protein levels in single living cells [5]. Cells expressing PQR constructs can be noninvasively imaged over time using standard fluorescence microscopy, allowing for other biological assays matched at the single-cell level, such as sequencing, mRNA expression analysis and pharmacological manipulations. For example, we used single-cell protein and mRNA quantification in single cells from human and mouse to determine the covariation between mRNA expression and protein amounts. We found that mRNA expression is a weak predictor of protein translation [5]. Recently, simultaneous measurement of RNA and protein was also performed in single cells using microfluidics, and a high heterogeneity and poor mRNA-to-protein correlations were also observed [9–11]. Current RNA analysis techniques such as RNA-Seq and microarrays, while amenable to single cells, are still costly and require deep coverage and sufficiently long reads of sequenced areas in order to accurately produce the sequence; particularly for transcripts expressed at low abundance [12].

Therefore, despite recent efforts to profile global gene expression from single cells, straightforward and inexpensive protocols are still not available. Here, we describe methods and protocols for fast and accessible quantification of gene expression by measuring both its absolute mRNA transcript numbers and level of protein translation in the same cell (Figure 1B). This tool can be used by any researcher with access to a standard ▶

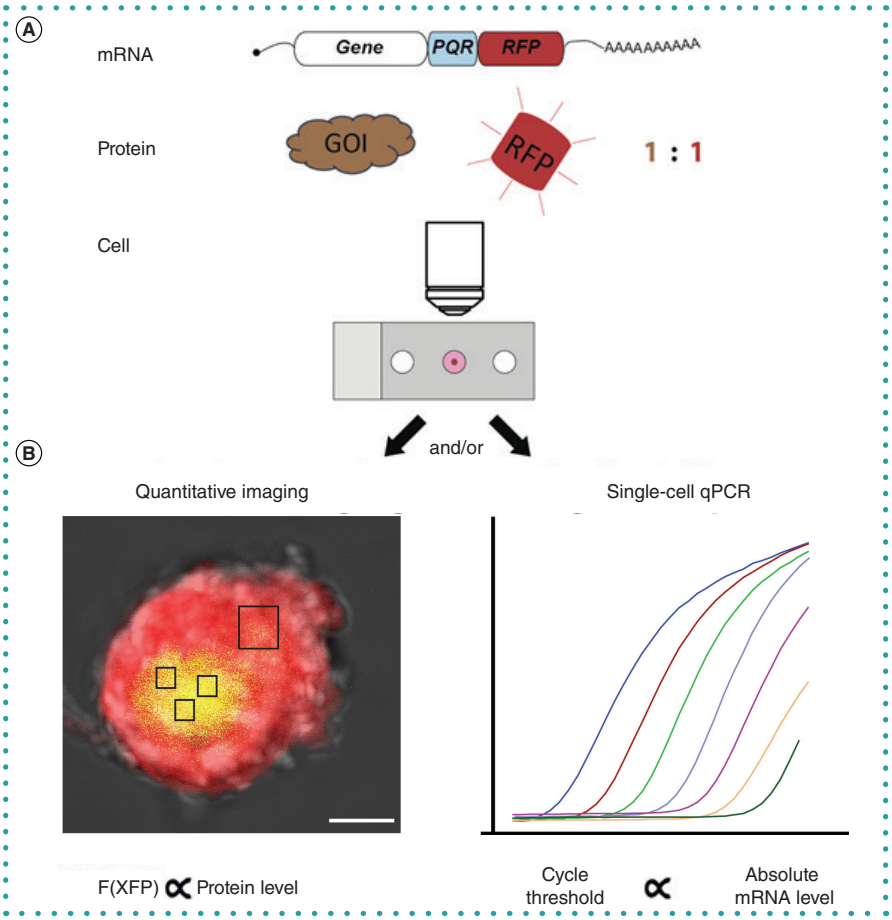

Figure 1. Workflow of protein and mRNA measurement from the same cell. (A) Insertion of a PQR reporter between a fluorescent reporter (*RFP*) and a GOI results in the stoichiometric cotranslation of a fluorescent reporter that can be used to quantify endogenous protein production levels. PQR constructs allow for one molecule of *RFP* to be produced for each molecule of protein of interest translated. Since the fluorescence output of GFP is directly proportional to its concentration, the fluorescence intensity of the cell can be used to determine the level of production of the protein of interest. **(B)** Quantitative fluorescence intensity measurement from regions of interest within the cell can be used to quantitate the production level of the upstream protein. Following imaging, the same cell is lysed for total RNA extraction and absolute mRNA abundance quantification using single-cell qPCR. Black boxes are representative regions of interest used for pixel intensity quantification.
GOI: Gene of interest; PQR: Protein quantitation ratioing.

▶ fluorescence microscope and real-time PCR instrument and can be easily applied to examine single cells obtained from sources such as dissociated tissue samples, FACS sorting or patient-derived stem cells. In addition, these methods are amenable to any cell type such as neurons, immune cells, immortalized cell lines in adherent or suspension form, and dissociated animal cells. Multiple genes with vastly different expression properties can be assayed simultaneously from the same cell, allowing the examination of gene co-regulation at both the transcriptional and translational levels.

Compared with existing single-cell protein or single-cell mRNA assays, our protocol requires 2.5 h from start to finish, is relatively inexpensive and can be performed using common laboratory tools.

MATERIALS & METHODS

Detailed reagents, protocols, experimental setup and troubleshooting information are presented in the supplementary information file.

Experimental design

We describe a method to first measure the level of protein abundance using a PQR fluorescent reporter, and then quantify the absolute mRNA copy number of that gene in a single cell (Figure 1B). As a source of single cells, we chose the antibody-producing monoclonal hybridoma mouse cell line 22c10 because they are large, easy to manipulate, and constantly secrete high levels of antibody containing kappa-isotype immunoglobulin light chains [13]. The insertion of a *PQR–RFP* reporter after the constant exon of the kappa light chain gene ensures the production of one molecule of *RFP* with each kappa light chain protein molecule translated (i.e., an antibody production reporter) (Figure 2A).

By contrast, we also quantified the *RPL13a* gene, which encodes for ribosomal protein L13a. *RPL13a* is expressed in all eukaryotic cells at consistent levels, and is routinely used as a reference 'housekeeping' gene for normalization of quantitative mRNA and protein measurements [14–16]. The quantification of *RPL13a* by the insertion of a *PQR–GFPnols* reporter at the end of its coding sequence (Figure 2A) can therefore be used as a measure of a cell's individual transcriptional and translational status [5].

Here, we take advantage of the differences between *IgK* and *RPL13a* expression to highlight how this technique can be used to profile the transcriptional and translational landscape of multiple genes simultaneously at single-cell resolution (Figure 2B). To generate 22c10 cells carrying PQR reporters at both the endogenous *RPL13a* and *IgK* loci, we first established a genome-edited cell line with a *PQR–GFPnols* at the *RPL13a* locus, and then targeted the *IgK* locus with a *PQR-RFP* reporter. The nucleolar (*nols*) targeting sequence on GFP in *RPL13a*-edited cells sequesters it into the nucleolus, a cellular organelle 100-times smaller than the cytoplasm in volume [17]. Sequestering fluorescent proteins into small cellular organelles increases their local concentration to produce brighter signals that facilitate fluorescence quantification and cell manipulation [5]. The colors of the fluorescent proteins can also be swapped, for example starting with an *IgK–PQR–GFP* edited cell line first (Supplementary Figure 1B).

We also apply this method on cells dissociated from genome-edited *Drosophila* that have a different color PQR reporter inserted into each parental allele to examine the relationship between paternal and maternal mRNA and protein expression in each cell.

Detailed information and protocols on the generation of PQR-edited single cells, quantitative imaging, mRNA reverse transcription, qPCR and data analysis are presented in the Supplementary file.

Cell culture

Genome-edited 22c10 cells were cultured at 37°C under 5% CO_2 in H-Cell (22c10) or DMEM (HEK293T), supplemented with 100 U/ml penicillin and 100 μg/ml streptomycin. For genome editing experiments, 800 ng of *CRISPR-Cas9* plasmid DNA were co-transfected with 800 ng of repair template circular plasmid in 12-well plates. After 2–7 days, fluorescent cells were manually isolated into single cell clones and expanded before verification of correct genome editing (see [5] for detailed methods). Double knockin cell lines were generated by first inserting a *PQR–GFPnols* reporter construct into the endogenous *RPL13a* locus to make a stable green fluorescent cell line. A *PQR–RFP* reporter construct was subsequently inserted into the kappa light chain immunoglobulin locus and cells that were fluorescent in both the green and red channels were selected for single-cell clonal expansion.

Protein quantitation reporter constructs

Protein quantitation reporter constructs were designed and constructed as described previously [5] and are available from Addgene.

Image acquisition & analysis

Fluorescence and brightfield microscopy was performed using a Zeiss AxioScope A1. All images were acquired at 1388 × 1040 pixels using a 40× water objective, N.A. 1.0 (epifluorescence) (Supplementary figure 2D). Fluorescence emission was detected using a charge-coupled device (CCD) camera (MRm). All image acquisition parameters were fixed for each imaging channel for exposure time, excitation intensity and gain. Cells that were dimmer or brighter than the fixed initial acquisition dynamic range were not included for analysis.

Images were selected for analysis based on identification of single healthy cells and low background. Fluorescence pixel intensities were measured in several random regions of interest (ROIs) within the cell cytoplasm (*IgK*) or nucleolus (*RPL13a*) using ImageJ. Average pixel intensities were calculated from five ROIs of 10 × 10 pixels for measurements within the cytoplasm and 5 × 5 pixels for measurements within nucleoli (Figure 2B). All signal intensities were background subtracted from the average of three 10 × 10 ROIs surrounding the cell.

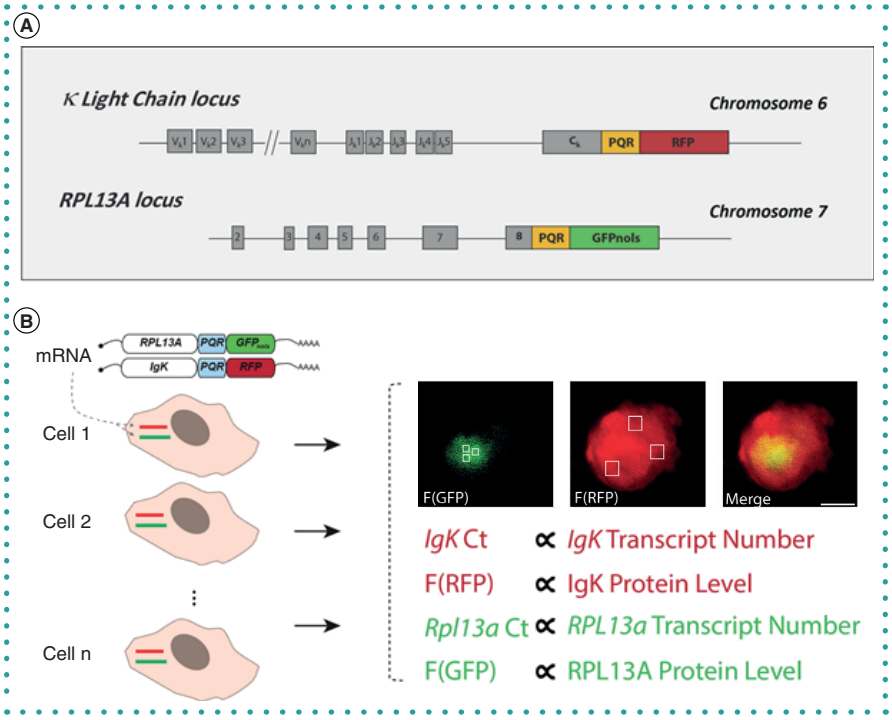

Figure 2. Protein and mRNA measurement for multiple genes in a single cell. (A) The fluorescent reporters (approximately 800 bp in size) are inserted in-frame immediately upstream of the stop codon, preserving the endogenous 5′ and 3′ untranslated regions and the native coding sequence. PQR constructs carrying an *RFP* and a GFP*nols* reporter were inserted into the endogenous loci of *RPL13a* and *IgK*, respectively, in 22c10 cells. **(B)** The transcript numbers and protein levels of both genes from the same cell can be obtained using single-cell qPCR and quantitative imaging. Representative example of a double knock-in 22c10 cell expressing *PQR–GFPnols* from the endogenous *RPL13a* locus and *PQR–RFP* from the endogenous *IgK* locus. White boxes are representative regions of interest used for pixel intensity quantification. Boxes placed in the nucleolus were used to quantify GFP (*RPL13a*) levels, and boxes placed in the cytoplasm were used to determine *RFP* (*IgK*) levels. PQR: Protein quantitation ratioing.

Real-time quantitative PCR

Detailed information and protocols describing single-cell isolation, RNA extraction, reverse-transcription and qPCR are presented as supplementary information. Briefly, total RNA was extracted from single cells using a modified Trizol-based protocol, and resuspended in 6 μl of ddH_20. Reverse-transcription reactions were performed with gene-specific cocktails (0.1 μM final concentration of each primer) using Superscript IV reverse polymerase (Thermo Fisher, MA, USA). 6 μl of cDNA was used as input template for real-time PCR using the TaqMan Fast Advanced Mastermix (Thermo Fisher). Real-time amplification was measured using the StepOnePlus real-time PCR system (Thermo Fisher) and cycle threshold values were calculated using StepOne software (Thermo Fisher). Absolute quantification of *RPL13a* and *IgK* mRNA copy numbers was determined using standard curves generated with synthesized oligonucleotide standards containing the *RPL13a–PQR–XFP* and *IgK–PQR–XFP* target. Primers and double-quenched FAM/ZEN/IowaBlackFQ probes were purchased from Integrated DNA Technologies (IA, USA) (all primer and probe sequences are shown in Table 1). See supplementary information for replicates and controls.

Statistical analysis

Linear correlations were calculated by fitting the data to a simple linear regression model, with the coefficient of determination, R^2. We tested the null ▸

TABLE 1. Sequences of primers and probes used.

	Forward primer	Reverse primer	RT primer	qPCR probe
Mouse *RPL13a*	TCCCTCCACCCTATGACAAG	GTCACTGCCTGG-TACTTCC	GCAGCCCTGCTACT-CATTTTC	GATGTAGAA-GAAAACCCTGGACC
Human *RPL13a*	TGTTTGACGGCATCCCAC	CTGTCACTGCCTGG-TACTTC	CTGCTGGCCACATTT-TATGTC	CTTCAGACGCAC-GACCTTGAGGG
IgK	AGTGGAAGATTGATGGCAGTG	CTGTCTTTGCTGTCCT-GATCA	GGTG-GATTTCAGGGCAACTA	ACAAAATGGCGTCCT-GAACAGTTGG
Mouse *RPL13a* outside of homology arms	CGGGTTGCTAACCTGGAATA	CAGTCTCCAT-CAAGGGGAAA		
IgK outside of homology arms		TAACTGGGGGAAGGGA-CACT		

hypothesis that the variables were independent of each other and that the true R^2 value was 0.

RESULTS & DISCUSSION

The PQR fluorescent reporters are translated stoichiometrically each time *RPL13a* or *IgK* protein molecules are produced. The fluorescence intensity of the cell (in arbitrary units) is proportional to the concentration of fluorescent reporter, which correlates directly with the level of translation of the protein of interest. Therefore, the fluorescence intensity or brightness of the cell can be used as a measure of the amount of production or steady state level of the protein of interest [5]. Similarly, the cycle threshold of the real-time amplification reaction is proportional to the concentration of input nucleic acid, and this is used to quantify the relative or absolute levels of the target mRNA [18]. Measuring the fluorescence intensity of the cell and Ct values from the qPCR amplification can therefore be used to assay the gene expression of any gene(s) from the same cell, and its variation between cells at both the transcriptional and translational levels.

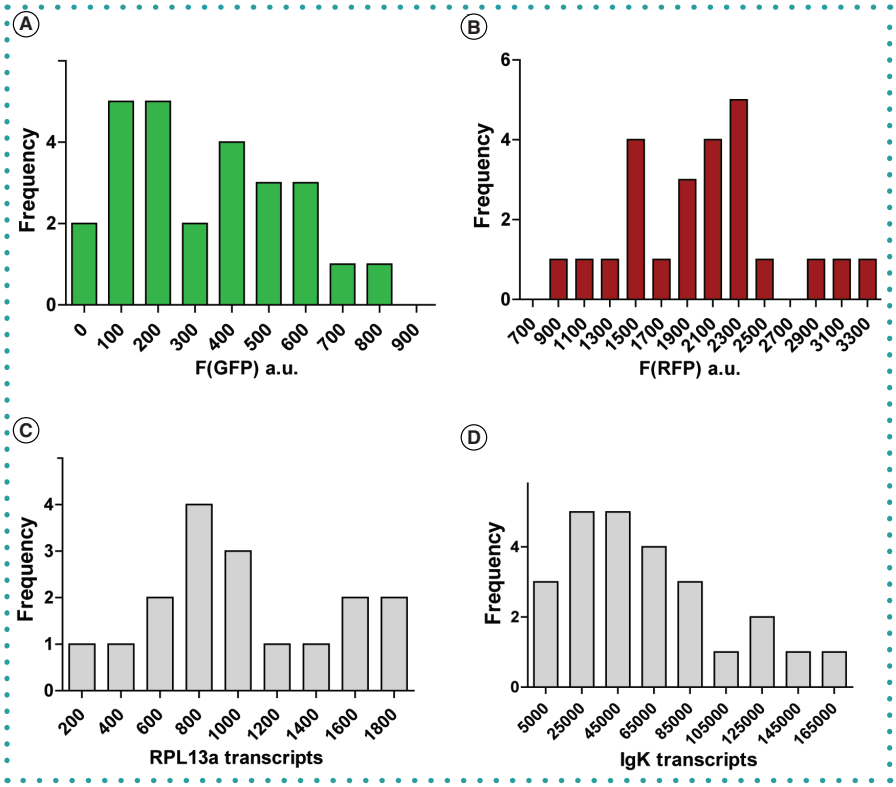

Figure 3. Endogenous RNA and protein quantification from single cells. (A) GFP fluorescence intensity from single genome-edited 22c10 cells shows a moderate distribution; n = 26. **(B)** *RFP* fluorescence intensities were higher and clustered more, indicating consistently high level *IgK* production; n = 24. **(C)** Frequency distributions of absolute *RPL13a* mRNA numbers shows moderate expression of the *RPL13a* gene; n = 17. **(D)** Absolute *IgK* mRNA levels were relatively higher, indicating high expression of the *IgK* gene compared to *RPL13a*; n = 24. a.u.: Arbitrary units; GFP: Green fluorescent protein; RFP: Red fluorescent protein.

To determine the levels of *IgK* and *RPL13a* protein in single 22c10 cells, we used CRISPR-Cas9 to generate a cell line carrying a *PQR–RFP* and *PQR–GFPnols* insertion at the endogenous *IgK* and *RPL13a* genomic loci, respectively. Single cells with both red and green fluorescence were isolated to derive single-cell clones. The level of expression of the PQR fluorescent reporter correlates with the expression of the target gene and therefore the relative levels of *IgK* and *RPL13a* protein were determined by quantifying the nucleolar GFP fluorescence and cytoplasmic *RFP* fluorescence intensities from a single cell, respectively. The same cell was then directly lysed and the absolute abundance of *IgK* and *RPL13a* mRNA transcripts were determined using quantitative real-time PCR (Figure 3).

From quantitative imaging of single 22c10 cells, we found that *RPL13a* protein levels, as measured by nucleolar GFP fluorescence intensity, ranged from 91 to 842 arbitrary units (a.u.) with an average of 364 ± 205 (mean ± SD; n = 26). By contrast, cytoplasmic *RFP* fluorescence intensities ranged from 936 to 3263 a.u, with an average of 2004 ± 619 a.u (n = 24) (Figure 3B).

Using a standard curve of serially diluted known amounts of *IgK* and *RPL13a* synthetic oligonucleotide DNA templates, the absolute number of *IgK* and *RPL13a* mRNA molecules was determined (Figure 3C & D). We found that the number of *RPL13a* mRNA transcripts

in single 22c10 cells ranged from 287 to 3023 transcripts, with an average of 1156 mRNA transcripts (Figure 3C). Similarly, *IgK* mRNA amounts ranged from 2420 to 159,000 transcripts, with an average of 63,835 IgK mRNA transcripts per cell (Figure 3D) (See Supplementary Figure 3B and Supplementary Table 1). This is to be expected as 22c10 cells are designed and selected to express high levels of kappa light chain [19,20], while RPL13a is a non-essential housekeeping gene [21].

To determine the relationship between gene expression at the transcriptional and translational levels, we analyzed the correlation between the mRNA and protein levels of *IgK* and *RPL13a* and found interesting differences that shed light on the variety of mRNA-to-protein relationships that exists for different genes, and the importance of examining gene expression at both the mRNA and protein level. For example, *RPL13a* mRNA levels were not correlated with RPL13a protein levels, with a correlation coefficient of 0.1 ($p > 0.005$; $n = 26$) (Figures 4A & E). In sharp contrast, *IgK* mRNA levels were a better predictor of *IgK* protein levels, with a correlation coefficient of 0.44 ($p < 0.005$; $n = 24$) (Figures 4B & E), which is similar to the global correlation between mRNA and protein abundances [22–24].

To determine whether levels of *RPL13a* protein co-varied with *IgK* protein, the red and green fluorescence intensities in single 22c10 cells were compared. We found no significant linear correlation ($R^2 = 0.14$; $p > 0.05$; $n = 24$) (Figure 4C). By contrast, to determine whether the expression of *IgK* and *RPL13a* mRNA might be linked, we compared absolute *IgK* and *RPL13a* transcript numbers. We found that *RPL13a* transcript numbers were a better predictor of *IgK* mRNA levels ($R^2 = 0.3$; $p < 0.05$; $n = 17$), compared with protein levels (Figure 4D). Time lapse imaging of *RPL13a* and *IgK* protein synthesis dynamics would reveal if there is a time-varying correlation between the two, given that *RPL13a* is itself involved in protein synthesis.

To determine how *RPL13a* mRNA and protein levels are correlated in human cell lines, we generated human embryonic kidney 293 (HEK293) cells carrying a *PQR–GFP* insertion at the endogenous human *RPL13a* locus. Using the same approach, we found that h*RPL13a* protein levels ranged

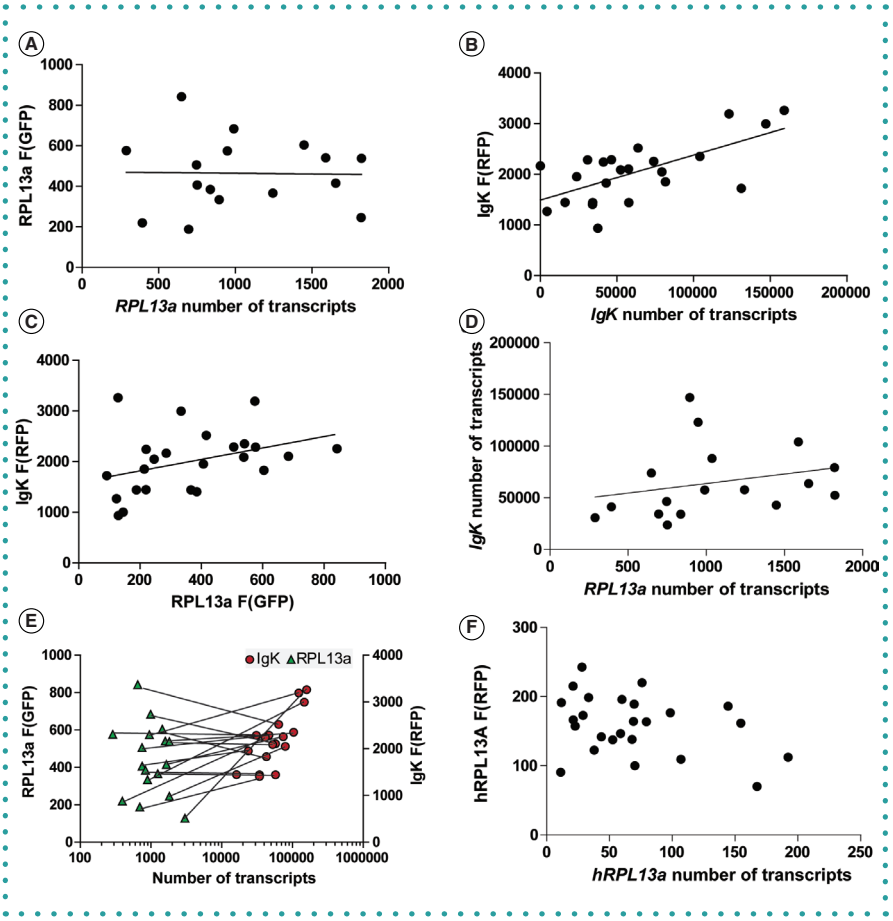

Figure 4. Protein and mRNA relationships between multiple genes in single cells. (A) *RPL13a* mRNA levels did not correlate with green fluorescence intensities indicating *RPL13a* mRNA is a weak predictor of *RPL13a* protein production ($R^2 = 0.0$; $p > 0.05$; $n = 17$). **(B)** *IgK* mRNA levels had a moderate correlation with red fluorescence intensities ($R^2 = 0.44$; $p < 0.05$; $n = 24$). **(C & D)** *IgK* and *RPL13a* exhibit nonsignificant correlations at both the protein level ($R^2 = 0.14$; $p > 0.05$; $n = 24$), and at the mRNA level ($R^2 = 0.06$; $p > 0.05$; $n = 17$). **(E)** Examination of mRNA and protein levels of multiple genes simultaneously from the same cell allows the co-correlation of mRNA and protein levels. Black lines connect data obtained from the same cell ($n = 17$). **(F)** Human *RPL13a* (h*RPL13a*) mRNA levels were also a poor predictor of red fluorescent intensities ($R^2 = 0.12$; $p > 0.05$; $n = 25$), indicating a similar kind of relationship between *RPL13a* mRNA and protein across the mouse and human genomes.

from 60 to 240 a.u. (compared with 91 to 842 a.u, for mouse *RPL13a*), and absolute *hRPL13a* mRNA transcript numbers ranged from 11 to 192 (compared with 287 to 3023 mouse *RPL13a* transcripts), resulting in a similarly poor inverse correlation between *hRPL13a* mRNA and protein levels ($R^2 = 0.12$; $p > 0.05$; $n = 25$) (Figure 4F). These results demonstrate similar mRNA–protein relationships for *RPL13a* between the human and mouse genomes.

Using the genome-edited trans-heterozygous *RPL13a–PQR–RFPnols/RPL13a–PQR–BFPnols* flies, we can track both the paternal and maternal *RPL13a* allele expression using *RFPnols* and *BFPnols* RNA and protein, respectively (Figure 5A). Using the approach described above, we determined that single epidermal cells dissociated from whole flies contained maternal *RPL13a* transcript numbers that ranged from 26 to 5565 and paternal *RPL13a* transcripts that ranged from 27 to 4753 (Figure 5B). Translation of the paternal allele, as measured by red fluorescence intensities; produced protein levels that ranged from 44 to 959 a.u. and translation of the maternal allele produced blue fluorescence intensities that ranged from 15 to 399 a.u. ($R^2 = 0.02$; $p > 0.05$; $n = 30$ for paternal *RPL13a* and $R^2 = 0.003$; $p > 0.05$; $n = 33$ for maternal *RPL13a*) (Figure 5B). In cells dissociated from a homozygous fly expressing *PQR–RFPnols*, *RPL13a* transcript numbers ▶

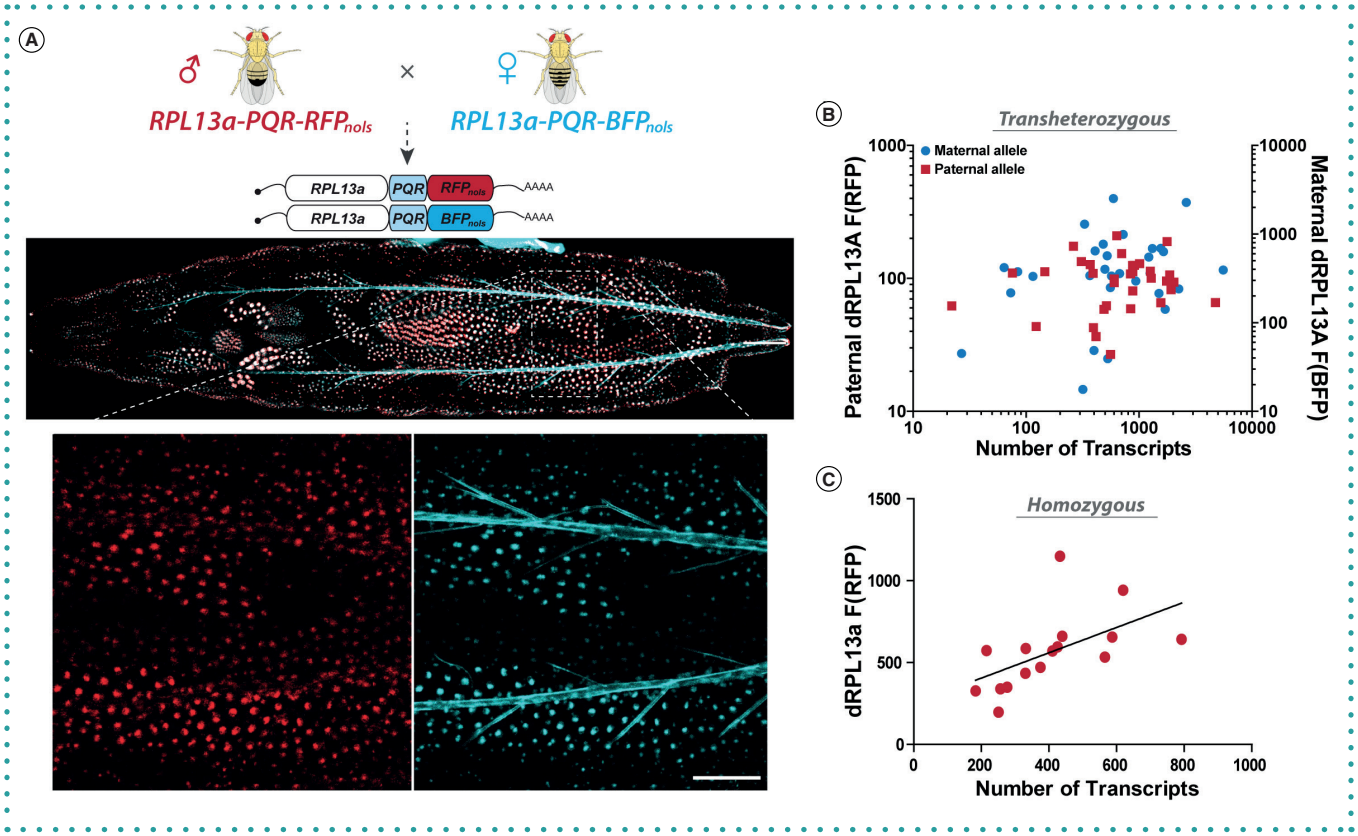

Figure 5. Tracking contribution of parental alleles at the mRNA and protein level. (A) Transheterozygous *RPL13a* knock-in flies co-express *PQR–RFPnols* each time the paternal *RPL13a* allele is transcribed, and *PQR–BFPnols* for the maternal allele expression. Every cell that expresses *RPL13a* will then produce nucleolar red and blue fluorescence. Trachea tubes are auto-fluorescent in the blue channel. **(B)** Examination of mRNA–protein relationships for both parental *RPL13a* alleles in single cells ($R^2 = 0.02$; $p > 0.05$; $n = 30$ for paternal *RPL13a* and $R^2 = 0.003$; $p > 0.05$; $n = 33$ for maternal *RPL13a*). **(C)** *Drosophila RPL13a* mRNA levels can predict *RPL13a* protein levels with 30% accuracy in *PQR–RFPnols* homozygous flies ($R^2 = 0.3$; $p < 0.05$; $n = 16$).

▶ ranged from 183 to 791 transcripts, while red fluorescence intensities ranged from 196 to 1149 a.u., which resulted in a correlation of $R^2 = 0.3$ ($p < 0.05$; $n = 16$) (Figure 5C). Consistent with our findings in the mouse and human genomes, *Drosophila RPL13a* protein levels could be predicted from absolute mRNA transcript levels with at best 30% accuracy.

The genome-wide correlation between mRNA and protein levels in cell populations is recognized to be poor, with only 40% of the variance accounted for between mRNA and protein levels [24]. This is typically attributed to regulatory mechanisms that can independently affect each step of gene expression. Measuring and correlating the levels of *IgK* and *RPL13a* mRNA and protein from single 22c10 cells shows exactly how different genes can have different mRNA–protein relationship dynamics: *IgK* mRNA level was a better predictor of *IgK* protein levels ($R^2 = 0.4$; $p < 0.05$; $n = 24$), compared to *RPL13a* mRNA to protein levels ($R^2 = 0.1$; $p > 0.05$; $n = 17$) (Figure 4E). In addition, *RPL13a* mRNA was more likely to co-vary with other mRNAs, compared with *RPL13a* protein variation (Figures 4C & E).

This approach also allows the examination of relationships between the expression of different genes and their products. Since this technique yields two metrics per gene, assaying multiple genes allows the co-correlation of mRNA and protein levels across different genes. This can be used to study the regulation and differential expression of mRNAs and proteins of multiple genes at the single-cell level. One analogous example is the use of housekeeping genes for the normalization of quantitative mRNA and protein measurements. In this case changes in *IgK* mRNA or protein levels may be normalized to the mRNA and proteins levels of *RPL13a*, a commonly used housekeeping gene for normalization of expression. For example, this knock-in 22c10 cell line can be experimentally manipulated to screen for cells that exclusively upregulate the expression of *IgK* antibodies, without affecting *RPL13a*. Similarly, this technique can be used to examine whether different genes are co-regulated within a single cell, under different treatment or disease conditions.

The method can be improved by faster collection and imaging of cells using automated or microfluidic chambers and using nonprecipitation approaches to isolate the total RNA. However, in its present form the protocol requires 5 days to perform CRISPR experiments and initiate clonal selection of cells carrying PQR reporters (Supplementary Figure 1A), and then 4 h from start of imaging to qPCR amplification (Supplementary Figure 2). In addition, the protocol does not require uncommon reagents or equipment and is accessible to a wide range of researchers. Because of the small quantity of starting template, certain low copy-number genes may fall below the detection range of the reverse-transcriptase or PCR polymerase

enzymes, and the success and quality of the amplification depends on the optimization of the primer and probe conditions, and the quality of the template. With 85% efficiency primers, our results show that the average amplification failure rate is around two to three in ten cells, and is target-dependent (nine failed *RPL13a* reactions vs 0 failed *IgK* reactions). Such results can be explained by variability in the RT step between *IgK* and *RPL13a* RT primers and by the substantially lower number of *RPL13a* transcripts (mean = 1156 transcripts) as compared with *IgK* (mean = 61,282 transcripts). The failure rate may be improved with more efficient primers, and this further increases the reproducibility of the assay (Supplementary Figure 3; Troubleshooting).

Analyzing the expression of genes at both the transcriptional and translational levels from the same cell can reveal previously hidden mechanisms that produce cellular phenotypes from gene expression. In addition, insertion of PQR reporters into a disease-associated gene can be used to readout mRNA and protein responses to drugs or therapies. Finally, this protocol can easily be applied to determine how well mRNA levels of a gene of interest can serve as a proxy for protein abundance, such as in circadian controlled genes.

AUTHOR CONTRIBUTIONS

IK and BEC designed the experiments. IK carried out the experiments. IK and BEC analyzed the data and wrote the manuscript.

ACKNOWLEDGMENTS

The authors thank Dr Chiu-An Lo and Dr Farida Emran for assistance with experiments.

FINANCIAL & COMPETING INTERESTS DISCLOSURE

This work was supported by a grant from the Natural Sciences and Engineering Research Council of Canada (B.E.C.). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

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SUPPLEMENTARY DATA

To view the supplementary data that accompany this paper please visit the journal website at: www.future-science.com/doi/full/10.2144/btn-2018-0130

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